

two-molecule images (with realistic point spread functions and Poisson noise model) as input. These images were generated with known molecule positions, to test the predictions of QuickPALM against the actual images. Our analysis showed that the accuracy dropped significantly when molecules were less than one wavelength apart, as there was rejection of multiple molecules in close proximity. With our results, we were also able to show that localization bias occurred towards the center of each pixel, distorting the overall image. The result was confirmed by simulating a PALM experiment on the US Air Force Test Pattern. We also wrote our own molecule localization plug-in (for ImageJ) utilizing second-moments (as a rejection criterion) and a fast maximum likelihood estimator (for estimating molecular positions), to compare with QuickPALM. We were able to show that the new plug-in had improved precision and accuracy when using the same single-molecule and two-molecule images. We also showed that the maximum likelihood estimator had no bias towards the center of each pixel when processing the US Air Force Test Pattern.

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Revival of Prism-Based TIR Microscopy - Versatile Tracking of Fluorescent and Scattering Probes with NM-Precision

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Current single-molecule microscopy experiments mostly rely on the fluorescence signal of fluorescent proteins, organic dyes or quantum dots attached to proteins of interest. However, these probes suffer from certain limitations, namely limited number of photons before photobleaching, photon blinking, and fluorescence saturation. Consequently, the temporal and spatial resolution by which single molecules can be tracked is limited. Promising candidates for replacing fluorescent probes are gold nanoparticles (GNPs). GNPs exhibit a large scattering cross section in the optical spectrum due to plasmon resonance, provide long-term stability and allow for versatile surface chemistry. Furthermore, because generation of photons relies on elastic scattering, their emission rate does not saturate.

Here, we present a camera-based wide-field imaging technique for GNP-labeled proteins using a novel parabolic prism-type total-internal reflection (TIR) microscope. We demonstrate the advantages of GNPs over commonly used fluorescent probes and discuss the pros and cons of prism-type versus objective-type TIR microscopy. We demonstrate that prism-based TIR microscopy allows imaging of fluorescent and scattering probes with high signal-to-noise, excellent control over a wide range of incidence angles, and even illumination intensities within a field of view. When tracking surface-immobilized GNPs (40 nm diameter) we obtained two-dimensional localization precisions as good as 5 angstrom within 30 ms exposure time. We demonstrate localization experiments of kinesin-1 motors labeled with GNPs walking on surface-immobilized microtubules. When GNPs were bound to the tail or motor domains of kinesin-1 we found localization precisions of 3.6 nm and 1.9 nm within 30 ms exposure time, respectively. Furthermore GNP-loaded motors showed 8 nm stepping along microtubules at 3 μ M MgATP within image acquisition times of 15 ms. Our method allows for precise localization of biomolecules within short acquisition times over long time scales.

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The Nuclear Pore Complex as seen by DstORM

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One of the most complex molecular machines of cells is the nuclear pore complex (NPC), which controls all traffic of molecules in and out of the nucleus. Due to their importance also for cellular processes such as gene expression and cytoskeleton organization, the structure of NPCs has been studied extensively during the last decades mainly by electron microscopy. Here we use super-resolution imaging by direct stochastic optical reconstruction microscopy (dSTORM) to investigate the structure of NPCs in isolated *Xenopus laevis* oocyte nuclear envelopes with a lateral resolution of ~ 15 nm. Generating accumulated super-resolved images from hundreds of NPCs we reveal a diameter of the central NPC channel of 41 ± 7 nm and demonstrate that the integral membrane protein gp210 is distributed in an eightfold radial symmetry. Two-color dSTORM experiments emphasize the highly symmetric NPCs as ideal model structures to control the quality of chromatic aberration corrections and to test the capability and reliability of super-resolution imaging methods.

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Single Molecule Fluorescence Microscopy Reveals Neurite-Bound Amyloid-Beta Oligomers

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Oligomers of the 39- to 42- residue amyloid-beta peptide are implicated as synaptotoxic factors in Alzheimer's disease. However, the stoichiometric identity of the species which damages neurons and the mechanism by which this species interacts with cells remain unknown. Particles ranging in size from small oligomers to protofibrils may be causative factors in loss of normal synaptic function, a harbinger of Alzheimer's. While these changes may be mediated through interaction with a specific receptor, significant evidence also points to pore formation and catastrophic calcium leakage as a possible mechanism for the peptide's toxicity. Using single molecule microscopy, we demonstrated that monomers and small oligomers bind to living cells even at nanomolar concentrations and that these oligomers can grow following binding (Johnson, et al. 2011. PLoS ONE 6(8): e23970). Similar methods are being applied to determine the size distribution of amyloid-beta oligomers which bind to the neurites of primary hippocampal neurons and to explore how changes in local environment affect this distribution. At low (1-10) nanomolar concentrations, amyloid-beta binds to neurites primarily as monomers to hexamers. A small number of larger neurite-bound oligomers, constituting less than 10% of the total population, is also present. Like oligomers previously observed on the somas of neuroblastoma cells, these neurite-bound species are immobile on a time scale of minutes and are significantly larger than oligomers adsorbed to the coverslip at these concentrations. Studies on the effects of these oligomers on intracellular calcium fluctuations and spine density provide insight into how cell-bound amyloid-beta oligomers impact neuronal electrophysiology and synapse integrity over time. Finally, oligomer binding location on the neuronal process is explored by fluorescence colocalization studies, in an effort to determine whether oligomers exhibit preferences for specific binding sites on the membrane.

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Novel Photoconvertible Fluorescent Proteins for Super-Resolution Imaging

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Recently developed super-resolution imaging techniques have revolutionized the study of cellular ultrastructure at unprecedented resolution. Novel fluorescent probes with distinct and optimized properties have been developed for these "super-resolution" imaging. Here we report two engineered photoconvertible fluorescent protein variants that are suitable for use in single-molecular microscopy such as photoactivated localization microscopy (PALM) and stochastic optical reconstruction microscopy STORM. Compared to alternative photoconvertible proteins, the variants show fast maturation rate, improved lightness of the green state, and outstanding high precision single molecular localization.

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Quantification of Photoactivatable Fluorescent Proteins by PALM

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We present a single molecule method for counting photoactivatable proteins, mEos2 and Dendra2, within a diffraction limited area when using photo-activated localization microscopy (PALM). The intrinsic blinking of photoactivatable fluorescent proteins (PAFPs) mEos2 and Dendra2, leads to an over-counting error, which constitutes a major obstacle for their use as molecular counting tags. Here, we introduce a kinetic model to describe the photo-blinking of Dendra2 and mEos2 proteins and use the measured blinking parameters to address the over-counting error. Simultaneous photoactivation of multiple molecules is another source of error, but it leads to molecular undercounting, instead. We propose a novel photo-activation scheme that separates uniformly the activation of different molecules, thus helping to decrease the undercounting error. Our method quantifies the total counting error and minimizes it by balancing the over- and undercounting.

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Sparse Statistical Deconvolution for High-Density Localization Microscopy

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Super-resolution imaging based on single-molecule localization techniques can achieve image resolution of ~ 20 nm but requires that the simultaneously